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The Genetics of Melanoma in Fishes. VI. Mendelian Segregation of Melanophore Reaction Types In Embryos of a Melanomatous Mother.[1]

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(Text-figures 1-3).

The experimental production of melanomas in hybrid fishes, by genetic methods alone, was first discovered, not by geneticists nor oncologists, but by aquarists in 1912 and 1913. The early history of their discoveries has been pieced together by noting the details presented in the minutes of their aquarium society meetings. In their early efforts to develop new and more colorful varieties of fishes for their home aquaria these fish-breeders crossed the black-spotted platyfish, *Platypoecilus maculatus*, with the swordtail, *Xiphophorus hellerii*. The aquarists were successful in breeding strikingly beautiful hybrids but the black-spotted ones developed melanotic tumors. The fish hybridizers have continued making new combinations. One of the more recent developments has been the black-banded hybrid with either yellow or bright red back, popularly spoken of as the "tuxedo swordtail." The junior author bred some of these in his private aquarium; the mating record is given in the diagrams. One of the hybrids that developed a melanoma was turned over to the senior author for further study.

The Melanomatous Female and Its Brood.

When the black-banded, yellow-backed female swordtail hybrid was about 20 months old and 65 mm. long, it developed a melanoma measuring 12 mm. × 7 mm. × 4 mm. on her left side. A smaller tumor appeared on the other side. At the time of its death it was gravid and when it was dissected, 40 embryos were found. Their age is estimated to be about 25 days, assuming that the average gestation period is thirty days.

Under a binocular microscope, the embryos were sorted out into four genetic classes, as follows:

10, *St N*, gray-backed and well developed black bands.

9, *st N*, light colored backs and poorly developed black bands.

12, *St n*, completely gray, like the wild type of swordtail.

9, *st n*, extremely light in color over all.

This typical backcross ratio of 1:1:1:1 was expected on the basis of two independent factors: *N* for the black band, and *St* for many tiny black pigment cells which make the fish appear gray. Bellamy (1928) showed that the black-banded pattern of the platyfish may be referred to a sex-linked dominant gene, *N*, for "nigra." Gordon (1931) confirmed this and added that *St* was a dominant autosomal factor for many small melanophores, micromelanophores, which have a "stippling" effect. When a fish has both dominant factors, *St N* it is black-banded and gray-backed. When the fish is recessive for the stippling factor, *st N*, it is black-banded and gold-backed. The black band is composed of many large melanophores, macromelanophores, while the stipple pattern is made of micromelanophores. The smaller are not the young stages of the larger but are independently developed.

The Early Development of the Black Band.

It is significant that the four phenotypic categories, *St N*, *st N*, *St n*, *st n*, can be distinguished in hybrid fishes in embryonic stages. Gordon (1931) pointed out in his study of the development of genetic patterns in the platyfish that in pure species, the black-banded type, *N*, could not be distinguished until the birth of the fish and even at this comparatively late stage, the *N* pattern is represented by only one or two discrete macromelanophores. It is therefore

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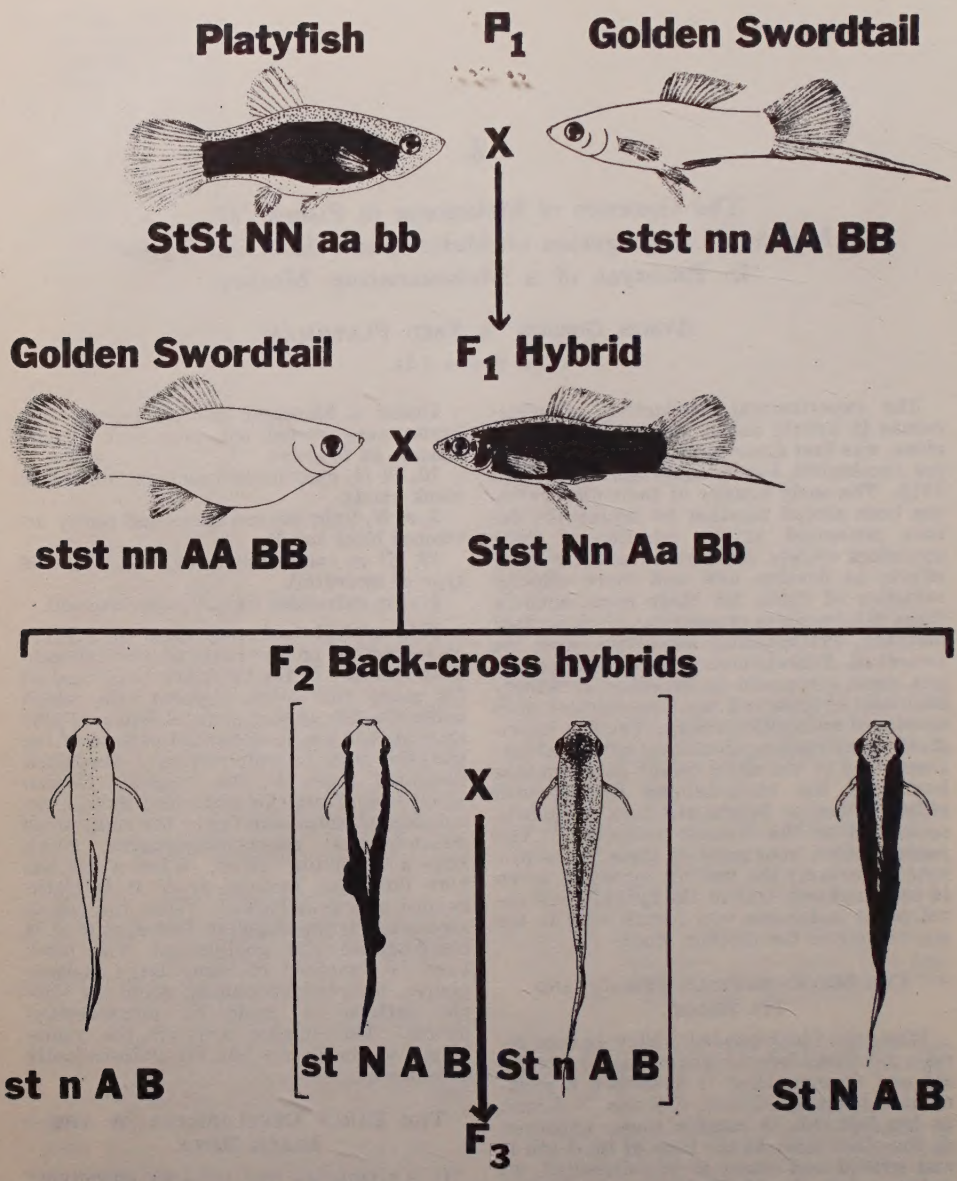
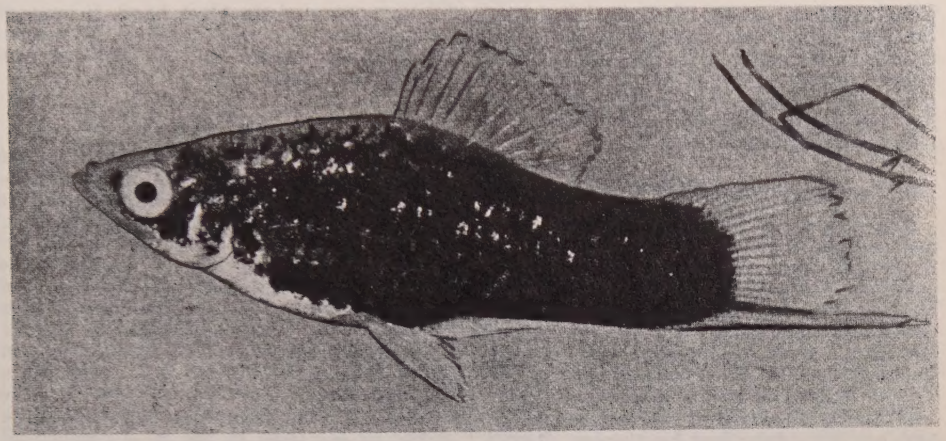
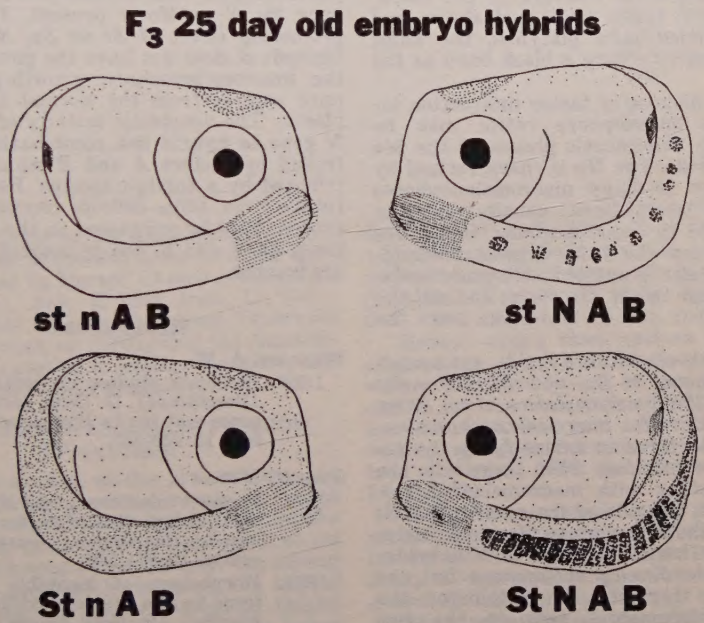


Text-fig. 1. The genetic history of the four types of embryos. A black-banded, gray-backed platyfish female, *StSt NN aa bb*, was mated to a golden swordtail male, *stst nn AA BB*. These are shown on the top. One of their black-banded, gray-backed sons, *Stst Nn Aa Bb*, was mated to a golden swordtail female, *stst nn AA BB;* the second mating is shown on the second line. The offspring of the second mating were of four types: *st n A B*, golden; *st N A B*, black-banded, golden-backed; *St n A B*, gray; and *St N A B*, black-banded, gray-backed. The four types are shown on the third row and the figures represent dorsal views of the adults. The mother of the embryos (shown in another Text-figure) is the black-banded, golden-backed type, *st N A B;* the father is the gray-type, *St n A B*. The father and mother are shown within the brackets in row three. The offspring of this mating may be seen in Text-fig. 3 on the opposite page.



Text-fig. 2. First generation hybrid. This hybrid is the product of mating a female black-banded *Platypoecilus maculatus* and the wild-type *Xiphophorus helleri*. In Text-fig. 1 it is represented on the second row to the right, under the legend F1 hybrid.



Text-fig. 3. Four genetic types of embryos. These embryos represent the offspring of the third generation. *st n A B* = golden; *st N A B* = black-banded, golden-backed; *St n A B* = gray; *St N A B* = black-banded, gray-backed. The black band is much better developed in the *St N A B* type than in the *st N A B* type, indicating an interaction of *St* and *N* genetic factors. (The authors wish to thank Mr. Jack Beckenstein for his aid in the preparation of these charts.)

quite apparent that the rate of growth of the macromelanophores is much accelerated in the hybrids. This marked increase in the rate of development is brought about by two genetic modifiers, *A* and *B* of the swordtail. Kosswig suggested this interpretation from his study of adult black-banded neoplastic hybrids. The change of the macromelano-

phore habit of growth from the normal to the pathological has also been analyzed by Gordon in his study of the *Sp* gene which is closely related to the *N* factor: *Sp* brings about the spotted pattern in pure platyfish and macromelanophore overgrowths in platyfish-swordtail hybrids.

The Influence of the *St* Gene Upon *N*.

The *N* gene is influenced by the micromelanophore factor *St* in addition to *A* and *B* and this effect can only be detected in the embryos. For instance, in the gold-backed, black-banded hybrid embryos, *st N*, the macromelanophores are far less numerous, and form a much weaker black band, than in the gray-backed, black-banded type *St N*. This is illustrated by the diagram.

A similar situation of interaction of factors has been described by Gordon (1928) in two varieties of the platyfish: the gold, spotted platyfish, *st Sp*, had far fewer macromelanophores than the gray, spotted type, *St Sp*. In this instance the differences persist throughout life whereas in the black-banded hybrid types mentioned above, and in black-banded pure platyfish, the adult *st N* type has as strong a black band as the *St N* type.

Another instance of factor interaction involving the melanophore ratios may be found in the two genetic phases of the *Mo* gene in hybrids. The *Mo* is characterized by the presence of many macromelanophores arranged in lateral lines; usually the body is orange-red in color. Gordon (1938) has pointed out that the *St Mo* type is outstandingly more heavily spotted with macromelanophores than the *st Mo* form; indeed, the *st Mo* has but one or two spots near the head.

In platyfish-swordtail hybrids, macromelanophores alone, *st Sp*, are able to evoke melanomas. Micromelanophores, alone, *St sp*, cannot do this. Yet micromelanophores exert a definite force in intensifying the severity of the neoplasm when these cells are present together with macromelanophores. For instance, hybrids of the constitution *St Sp* develop their tumors earlier and faster than *st Sp*. This is also true in *N* hybrids: *st N* hybrids develop melanomas but the *St N* develop them first. An insight into the changing relationships between the two types of melanophores of a melanotic hybrid may be had by comparing the melanophores in *st N* embryos and in adults. In the *st N* embryo some micromelanophores are found along the dorsal ridge of the back and extend over the meninges of the brain; the number of micromelanophores found is, of course, far less than in the *St N* embryo. In the *st N* adult, practically all the micromelanophores have been eliminated by the hypertropic activity of the macromelanophores in the making of the black band. It appears that as the *st N* hybrid develops, its macromelanophores usurp all the melanin-producing substances.

Summary.

The primary genic function of *St* is the formation of thousands of micromelanophores. This clothes the platyfish or the swordtail with a grayish coat of pigment. The secondary effect of the *St* gene is to accelerate the growth-promoting forces of the macromelanophores both in normal and neoplastic stages of their development. For example, more macromelanophores appear at birth of a hybrid in a *St Sp* than in a *st Sp*; more in a *St N* than in a *st N*. More macromelanophores are developed by the adult *St Sp* than by a *st Sp* platyfish; more in the *St Mo* than in the *st Mo* swordtail derivative.

Thus the *St* gene has a dual effect: first, in the production of many micromelanophores and second, in the stimulation of the production of macromelanophores when the gene *Sp*, *N* or *Mo* is present. The growth promoting effect of *St* on *Sp*, *N* or *Mo* is limited; *st* does not have the power to shift the macromelanophore growth-pattern in pure species from the normal to the neoplastic. The neoplastic activity of the *Sp* or *N* gene in hybrid fish combinations is controlled by factors *A* and *B* which are contributed by a foreign species. Perhaps at a future time, some definite physico-chemical process may be suggested in the activity of genes *St*, *A* and *B*, but at present such data are lacking.

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